

Difference of the effectiveness of rinsing with 0.2% *chlorhexidine* and 2.5% green tea solution (Tong Tji brand) of the number of *Streptococcus mutans* colonies in children's saliva aged 6-12 years

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ABSTRACT

The aim of this study was to determine differences in the effectiveness of rinsing with 0,2% chlorhexidine and 2,5% green tea solution in reducing the number of Streptococcus mutans. This study is quase experimental study with cross-over design. A total of 30 children aged 6-12 years old with poor OHI-S index. Saliva samples were collected before and after intervention. Rinsing intervention with chlorhexidine and green tea solution were given with wash-out period for 1 week. After intervention, saliva samples taken 15 minutes and 30 minutes after rinsing. Saliva samples were taken to the laboratory then the number of Streptococcus mutans count were evaluated. The statistical analyzed were performed by SPSS version 18.0 with ANOVA, t-paired and LSD test. There is no significant difference between chlorhexidine and green tea solution in reducing the number of Streptococcus mutans. chlorhexidine showed a greater reduction than green tea solution. However, chlorhexidine is a chemical mouthwash with some side effects while green tea is a natural substance that is safer for children.

Keywords: *chlorhexidine, green tea, Streptococcus mutans, effectiveness of rinsing*

INTRODUCTION

Until now, dental caries remains a major problem in the field of dentistry and became one of the most common chronic diseases among children. According to the Basic Health Research (RISKESDAS) in 2007 cited by Darwita, the prevalence of oral health problems in primary school children reached 72.1%.¹

One of oral pathogens that cause caries and found in the cariogenic biofilm or plaque is *Streptococcus mutans*. Many studies prove the close relationship between the number of *Streptococcus mutans* colonies in saliva with the prevalence of dental caries. Children with high caries rate has also increased *Streptococcus mutans* count.²

There are many ways of reducing the number of bacteria colonies in the oral cavity. One of them is mouthwash. In Indonesia, a mouthwash that most familiar to dental professionals for prevention of dental caries is *chlorhexidine*. *Chlorhexidine* is a broad-spectrum antimicrobial agent and has a bactericidal effect against all categories of microbes, including bacteria, fungi and viruses.

Fardal et al study cited by Kocak suggests that *chlorhexidine* shown to inhibit plaque formation, reduce gingival inflammation and prevent dental caries. Rindom et al study cited by Gupta suggested that rinsing with 10 ml of *chlorhexidine* 0.2% once daily can reduce 30-50% in the population of *Streptococcus mutans*. *Chlorhexidine* is most effective in reducing the number of *Streptococcus mutans*.^{3,4,5}

Over time, many studies have been done and found that herbal plants have great potential as drugs to prevent oral disease, such as green tea. Green tea can be used as a mouthwash to inhibit plaque formation and prevent dental caries, periodontal disease, halitosis and oral cancer. In addition, green tea can also prevent cardiovascular disease, stroke, obesity and cancer.^{6,7}

Bioactive components of green tea are able to influence the process of dental caries by inhibiting proliferation, acid production, metabolism, and enzyme activity of glucosyltransferase (GTF) of *Streptococcus mutans* and plaque. Signoretto research cited by Tehrani suggests that green tea consumption can inhibit plaque formation and decreases *Streptococcus mutans* and *Lactobacillus* in plaque and saliva. According to Cao Jin cited by Wijaya, catechins concentration 0.125% -1% showed decrease in the number of bacteria, plaque formation and total number of bacterial proteins and extracellular glucan. According to Wijaya and Samad, minimum inhibitory concentration of green tea against *Streptococcus mutans* was 2.5%, although the concentration of 1% have been able to inhibit the growth of *Streptococcus mutans*.^{6,8,9}

Previous study shown that green tea solution and *chlorhexidine* were effective in reduces the number of *Streptococcus mutans*. But study about the differences of these two materials has not been done. Based on these considerations, the researcher want to know whether a green tea solution have the same efficacy with *chlorhexidine* or different in reducing the number of *Streptococcus mutans*.

MATERIALS AND METHODS

This study was quase experimental with cross-over design that was held on 22 February-29 March 2013 in Inpres Hasanuddin University elementary school and Laboratory of Microbiology, Faculty of Medicine, University of Hasanuddin.

In this research, sample was taken from Inpres Hasanuddin University elementary school students aged 6-12 years old using *simple random sampling* method. The samples used were children aged 6-12 years with poor OHI-S index. Total sample are 30 (20 boys and 10 girls). Because this study used a cross-over design, total sample was 60 people. Each sample was given rinsing intervention with 0.2% *chlorhexidine* and 2.5% green tea solution.

Inclusion and Exclusion Criteria

The inclusion criteria were children with poor OHI-S index, have at least four teeth with carious lesion and willing to follow the procedures. The exclusion criteria were children with autoimmune diseases or other systemic diseases, took antibiotics consumption and not willing to follow the procedures.

Preparation of Mouthwashes

This study used these tools and materials: diagnostic set, nierbekken, glass, plastic pots, handschoen, incubator, test tubes, sterilization equipment, petri dish, green tea solution 2.5%, *chlorhexidine* mouthwash 0.2%, distilled water and Glucose Nutrient Agar (GNA) medium.

Green tea solution 2.5% were made by brewing 7.5 grams of green tea (Tong Tji brand) with 300 ml of distilled water 70-80 ° C. *Chlorhexidine* mouthwash 0.2% is mouthwash (Minosep brand) with 0.2% concentration.

Saliva Samples and Microbial Evaluation

Before the study conducted, oral hygiene examination was done using OHI-S index on prospective samples. The sample was children with poor OHI-S index. Sample was asked to not eat or drink for at least 2 hours before the procedure.

Saliva sampling were collected before rinsing intervention. Then intervention was given with *chlorhexidine* and green tea solution. Both of these materials are given with wash-out period of 1 week. Samples were instructed to rinse for 30 seconds. After rinsing, saliva samples were taken 15 minutes and 30 minutes after rinsing. Subsequently, samples were taken to the laboratory for *Streptococcus mutans* evaluation. Samples were diluted to 10^{-3} then isolated on GNA medium and incubated for 24 hours at 37 ° C. Then do the observations of bacteria and counting the number of *Streptococcus mutans*.

Statistical Method

The analyses were processed by SPSS 18.0, analyzed and tested statistically by ANOVA test and paired t-test, along with LSD test, with 95% confidence interval.

RESULTS

Table 1 shows the distribution characteristics of the sample with total of 30 people. However, because this study used cross-over design, total sample were 60 samples. The number of boys more than girls, with 20 boys (66.7%) and 10 girls (33.3%) with an average age around 8 years. An average value of oral hygiene sample is 3.70. The number of *Streptococcus mutans* colonies (CFU / ml) before intervention is 85.57. Meanwhile, after the intervention of *chlorhexidine*, after 15 minutes decreased to 43.80 and after 30 minutes decreased to 14.47, while the green tea group, the number of CFU after 15 minutes to 44.20 and after 30 minutes to 17.50.

Table 2 shows the effectiveness of each mouth rinse used in this study, *chlorhexidine* and green tea solution. Reduction in the number of *Streptococcus mutans* colonies were showed from before the intervention, 15 minutes after the intervention, and finally 30 minutes after the intervention. This applies to each mouthwash, both *chlorhexidine* and green tea. Based on the results of the ANOVA test, the value of $p = 0.000$ ($P < 0.05$) in both groups. This shows a decrease generated by each mouthwash is a significant reduction.

Table 3 shows the LSD test results of the number of *Streptococcus mutans* colonies based on time intervals after rinsing with *chlorhexidine* and green tea solution. This table is the result of LSD test after ANOVA test showed significant differences. At this table, the differences of each bacterial colony count by time intervals were described.

The decrease in the number of colonies by the whole time interval showed significant results

Table 1. Distribution characteristics of the samples

Characteristics of the samples	Frequency (n)	Percent (%)	Mean \pm SD
Sex			
Male	20	66,7	
Female	10	33,3	
Age			8.57 \pm 2.02
Oral hygiene score (OHI-S)			3.70 \pm 0.65
Number of <i>Streptococcus mutans</i> colonies before intervention (Pretest)			85.57 \pm 18.03
<i>Chlorhexidine</i> group	30	100	
Number of colonies in 15 minutes after intervention (Posttest 1)			43.80 \pm 15.62
Number of colonies in 30 minutes after intervention (Posttest 2)			14.47 \pm 8.85
Green tea solution group			
Number of colonies in 15 minutes after intervention (Posttest 1)			44.20 \pm 14.24
Number of colonies in 30 minutes after intervention (Posttest 2)			17.50 \pm 10.32

Table 2. Comparison of the effectiveness of each *chlorhexidine* and green tea solution based on the time interval

Mouthwash Group	CFU Pretest	CFU 15 minutes	CFU 30 minutes	<i>p-value</i>
	<i>Mean ± SD</i>	<i>Mean ± SD</i>	<i>Mean ± SD</i>	
<i>Chlorhexidine</i>	85.57 ± 18.03	43.80 ± 15.62	14.47 ± 8.85	0.000*
Green tea solution	85.57 ± 18.03	44.20 ± 14.24	17.50 ± 10.32	0.000*

*Repeated Analysis of Variance (ANOVA) test: $p < 0.05$; significant

Table 3. LSD test results of the number of colonies of *Streptococcus mutans* based on the time intervals after rinsing with *chlorhexidine* and green tea solution

Mouthwash Group	CFU <i>S.mutans</i>	Comparator	Mean Difference	p-value
<i>Chlorhexidine</i>	CFU pretest	CFU 15 menit	41.767	0.000*
		CFU 30 menit	71.100	0.000*
	CFU 15 minutes	CFU 30 menit	29.333	0.000*
Green tea solution	CFU pretest	CFU 15 menit	41.367	0.000*
		CFU 30 menit	68.067	0.000*
	CFU 15 minutes	CFU 30 menit	26.700	0.000*

*Pos Hoc Test: Least Significant Difference (LSD) test: $p < 0.05$: significant

Table 4 shows comparison of the effectiveness of *chlorhexidine* and green tea solution at intervals of 15 minutes and 30 minutes after rinsing. Once an individual basis, it is stated that the *chlorhexidine* and green tea solution effectively reduces the number of *Streptococcus mutans* colonies, then the table will compare these two mouthwashes, which one was better in reducing the number of *Streptococcus mutans* colonies. In table 4, it showed that after rinsing with *chlorhexidine* and green tea solution, at 15 minutes after rinsing, both mouthwash showed a similar results. In the *chlorhexidine* group, the number of colonies decreased to 43.80 CFU / ml, whereas the number of colonies in green tea group decreased to 44.20 CFU / ml. The difference are only 12.40 CFU / ml. Based on the results of statistical tests, obtained $p = 0.061$ ($P > 0.05$), meaning that the difference is not significant and can be considered the same. Meanwhile, after 30 minutes, *chlorhexidine* reduced the number of *Streptococcus mutans* colonies to 14.47, while the green tea solution is only reduced by about 17.50. There are differences of up to 3 CFU / ml at 30 minutes after rinsing. Based on the results of statistical tests, the value of $p = 0.656$ ($P > 0.05$), which means that there is no difference in the number of *Streptococcus mutans* colonies significantly between *chlorhexidine* mouthwash and green tea solution at intervals of 30 minutes after rinsing. However, *chlorhexidine* showed greater effect than green tea

Tabel 4. Comparison in the effectiveness of *chlorhexidine* and green tea solution at intervals 15 minutes and 30 minutes after rinsing intervention

Mouthwash Group	CFU Pretest	CFU 15 minutes	p-value	CFU 30 minutes	p-value
	Mean \pm SD	Mean \pm SD		Mean \pm SD	
<i>Chlorhexidine</i>	85.57 \pm 18.03	43.80 \pm 15.62	0.061**	14.47 \pm 8.85	0.656**
Green tea solution	85.57 \pm 18.03	44.20 \pm 14.24		17.50 \pm 10.32	

**Paired sample t-test: $p > 0.05$; not significant

DISCUSSION

Based on the results, there were significant effectiveness in decrease the number of *Streptococcus mutans* colonies based on the time interval with both mouthwashes. This shows that there is a significant reduction in the number of *Streptococcus mutans* colonies after rinsing with both

antimicrobial agents. It can be seen from the same phenomenon from both types of material, with a decrease in levels of *Streptococcus mutans* colonies after rinsing with *chlorhexidine* and green tea solution at 15 minutes and 30 minutes after intervention.

The effectiveness is suitable with previous studies that showed reduced number of *Streptococcus mutans* after rinsing with green tea solution. Content of green tea polyphenols, especially catechins can inhibit bacteria growth by damaging the bacterial cell wall. Minimum inhibitory concentration of green tea solution by Wijaya and Samad was 2.5%, according to the concentration of green tea used in this study. This concentrations showed significant antibacterial activity against *Streptococcus mutans*. Awadalla et al reported that gargling with green tea solution without sugar strongly inhibits the growth of *Streptococcus mutans* in plaque and saliva which is the major bacterial causes dental caries. Tehrani suggests that green tea solution showed more significant reduction of *Streptococcus mutans* and *Lactobacillus* in the children's saliva than sodium fluoride mouth rinses.^{6,9,10}

Polyphenols content in green tea is a disinfectant, antiseptic, and able to inhibit the growth of microorganisms, because the polyphenols have the ability of protein denaturation and damage cell membranes of microorganisms. In addition, the bioactive component of green tea is able to influence the process of dental caries by inhibiting the glucosyltransferase (GTF) that interfere with the attachment of bacteria to the enamel.^{6,11}

Effectiveness of *chlorhexidine* in reducing the number of *Streptococcus mutans* colonies is also suitable with previous study by Neeraja stating that *chlorhexidine* can reduce the number of *Streptococcus mutans* colonies in plaque and saliva. *Chlorhexidine* binds strongly to all the surface structure. When *chlorhexidine* binds to the cell wall surface, permeability of the cell will increase, will lead to rupture of the cell membrane, leading to osmotic imbalance with penetration into the cytoplasm, resulting in cell death.^{2,3}

The results also showed a difference in the effectiveness of *chlorhexidine* and green tea solution at intervals of 15 minutes and 30 minutes after rinsing. In the 15 minutes after rinsing, the result in both mouthwashes is almost the same. In the *chlorhexidine* group, the number of colonies decreased to 43.80 CFU / ml, while in the green tea group decreased to 44.20 CFU / ml. After 30 minutes, *chlorhexidine* reduced the number of colonies to 14:47 CFU / ml, while the green tea solution successfully reduced until 17:50 CFU / ml. This shows that there is no significant difference between *chlorhexidine* mouthwash and green tea solution in decrease the number of *Streptococcus mutans* colonies in the 15 minutes and 30 minutes after rinsing.

It has been shown that both mouthwashes are effective in reducing the number of *Streptococcus mutans* colonies, but *chlorhexidine* showed greater effect than green tea at intervals of 15 and 30 minutes after rinsing in children's saliva aged 6-12 years. However, there is no significant difference between *chlorhexidine* and green tea solution at 15 minutes and 30 minutes after rinsing. Noteworthy that *chlorhexidine* is a chemical mouthwash that has side effects as bitter taste and staining, while green tea is a natural substance that is safer for children. In line with Moghbel et al study cited by Kukreja that green tea solution is safe for children and pregnant women.^{12,13}

CONCLUSION

The results of the present study showed that there is no significant difference in effectiveness between the *chlorhexidine* and green tea solution to the number *Streptococcus mutans* colonies in children's saliva aged 6-12 years. *Chlorhexidine* showed greater effect than green tea. However, noteworthy that *chlorhexidine* is a chemical mouthwash with few side effects that can appear during use. While green tea is a natural substance that is safer for children.

SUGGESTION

Based on this conclusion, it is suggested that *chlorhexidine* and green tea can be used as a mouthwash for children because it has many benefits for oral health. Then need to conduct further study on the differences between the effectiveness of *chlorhexidine* or green tea solution with other natural materials. Also need to do more study to work with pharmaceutical to make a formulations of green tea mouthwash that is not bitter without reducing the content of pure green tea itself.

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